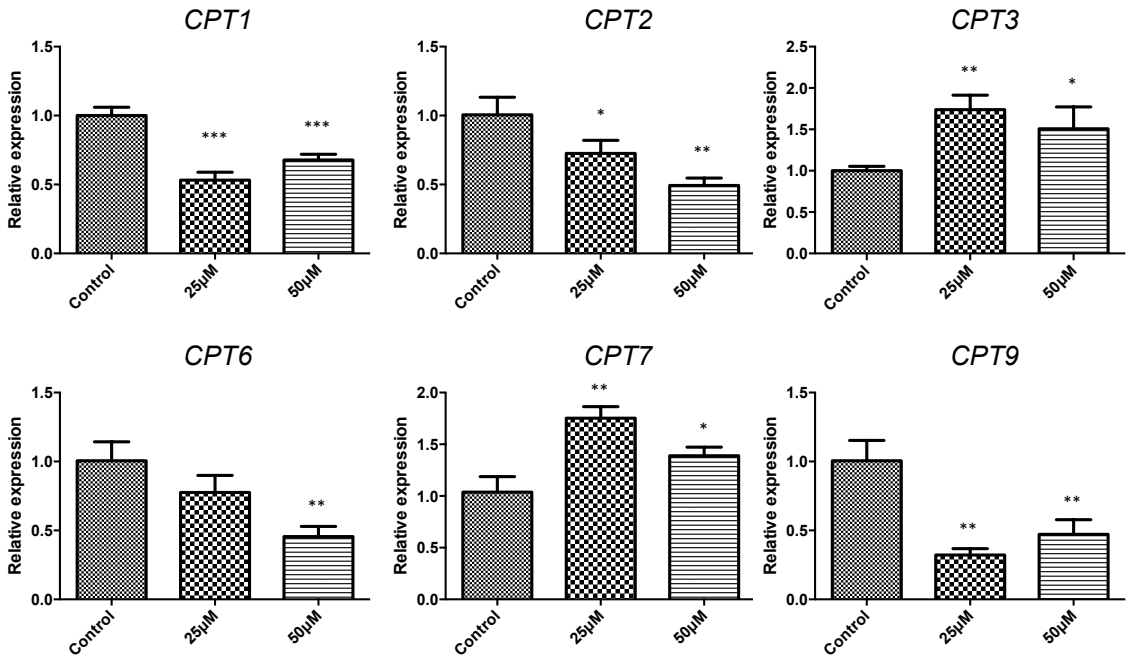
Supplementary Figure 1

Effect of cadmium chloride on dolichol accumulation and expression of CPT-encoding genes

When the hairy root growth medium was supplemented with cadmium salt (25 or 50 μM $CdCl_2$), dolichol content was moderately increased and reached respectively, 130% and 150% of control (Table 1). Similarly to sorbitol, cadmium salt modulated the composition of the dolichol family and had no statistically significant effect on the accumulation of phytosterols (Table 1).

$CdCl_2$ decreased the expression of four CPT-encoding genes, *CPT1, -2, -6* and *-9* (to approx. 40% of control) and elevated expression of *CPT3* and *-7* (approx. 180% of control for 25 μM $CdCl_2$). Interestingly, the expression of *CPT6* responds differentially to cadmium ions and sorbitol suggesting a unique role of CPT6 in the cell response to different environmental stresses.

Cadmium ions have been found to cause disarrangement of actin filaments and Ca^{2+} gradient, thereby inhibiting vesicular trafficking and consequently altering the cell wall organization (Wan and Zhang, 2012). Metabolomic studies on Arabidopsis plants treated with a cadmium salt have revealed increased levels of amino acids (alanine, proline, serine), sugars (sucrose, raffinose and trehalose) and other metabolites such as 4-aminobutyric acid and glycerol (Sun et al., 2010). Cadmium salt treatment produced contrasting effects on putrescine level – it was increased in oat and bean plants but decreased, together with spermidine, in the sunflower (Ramakrishna and Ravishankar, 2011). Another well studied group of metabolites affected by cadmium treatment are soluble phenolics, e.g., *p*-coumaric and ferulic acids found to be increased in mycorrhizal roots (Schützendübel and Polle, 2002). In contrast to the present report, cadmium salt treatment caused a significant increase of the concentration of phytosterols - campesterol, β-sitosterol and other isoprenoids such as α-tocopherol (Sun et al., 2010) in Arabidopsis. That finding underlines metabolic differences between various biological systems (here, whole plants vs. root culture) even when of a single species.



Supplementary Figure 1

Effect of cadmium chloride on expression level of *CPTs* in Arabidopsis hairy roots.

Supplementary Figure 2

Effect of oxidative stress on dolichol accumulation and expression of CPT-encoding genes

Oxidative stress is a common consequence of environmental stressors (Gechev and Hille, 2012) and production of reactive oxygen species (ROS) has been described as a general feature of various rhizotoxic treatments (Zhao et al., 2009). ROS act both as oxidative agents aggressively reacting with cellular components, and as signal transducers regulating plant development, hormone signaling, programmed cell death, and biotic and abiotic stress response and tolerance (Lin and Aarts, 2012).

To gain a deeper insight into the mechanisms responsible for the increased dolichol content upon sorbitol and cadmium salt stresses ROS signaling was mimicked by treating roots with oxidizing compounds – hydrogen peroxide (HP, 1 mM), methyl viologen (MV, 1 μM) or *tert*-butyl hydroperoxide (*t*BHP, 0.3 mM).

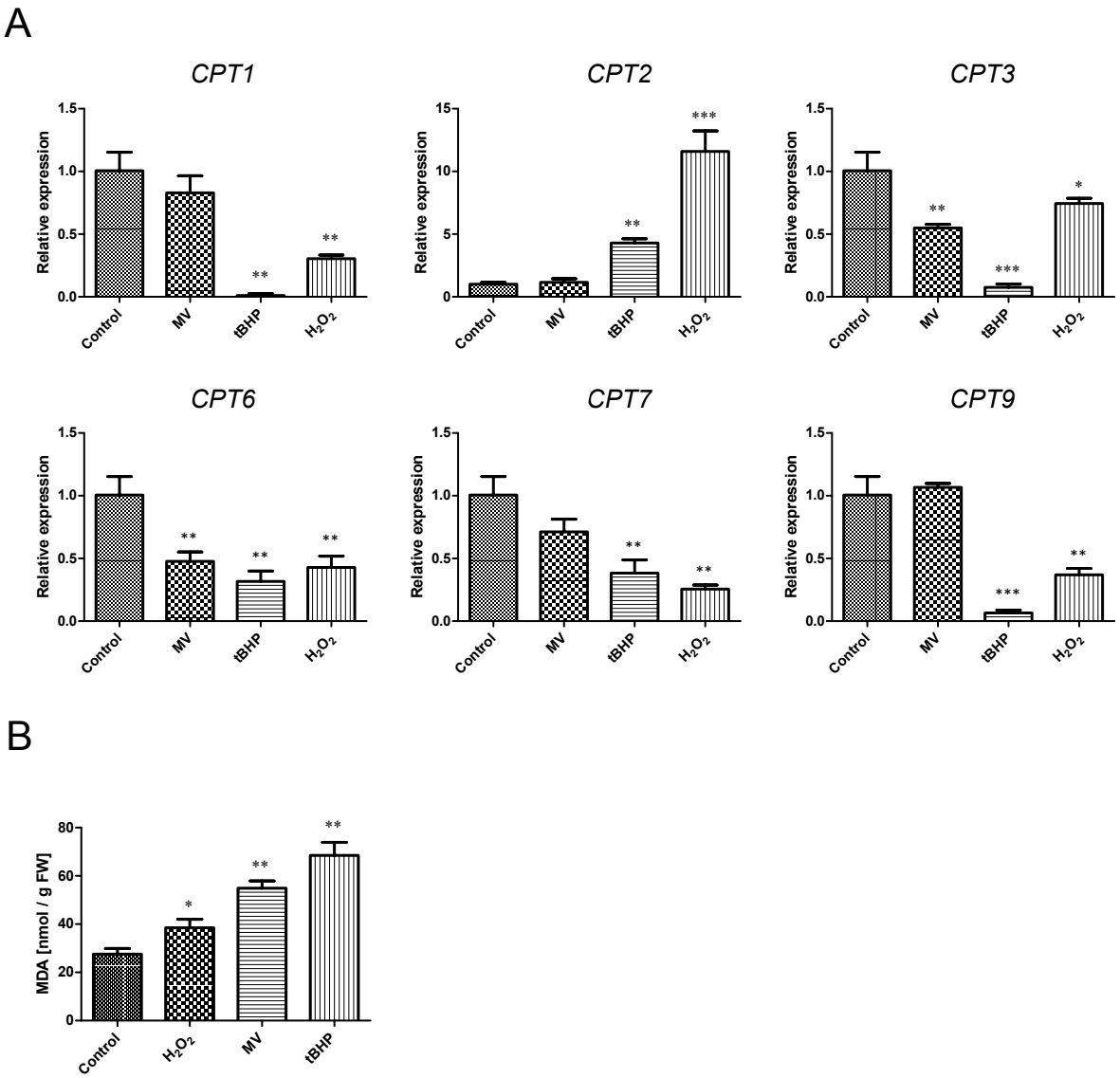
While the HP and *t*BHP treatments resulted in a modest increase of dolichol cellular level (in both cases to approx. 120% of control), MV did not cause a statistically significant change (approx. 90% of control) (Table 1).

In contrast, the sterol content showed profound changes upon tBHP and MV treatments, to 260% and 200% of the control, respectively, while HP had no effect (Table 1).

An increased expression of *CPT2* was observed upon the *t*BHP and HP treatment (8- and 12-fold, respectively), and decreased expression of three *CPT*s (*CPT1*, *-3* and *-9*). Methyl viologen slightly decreased the transcript level of all the *CPTs* (Supplementary Fig. 2A).

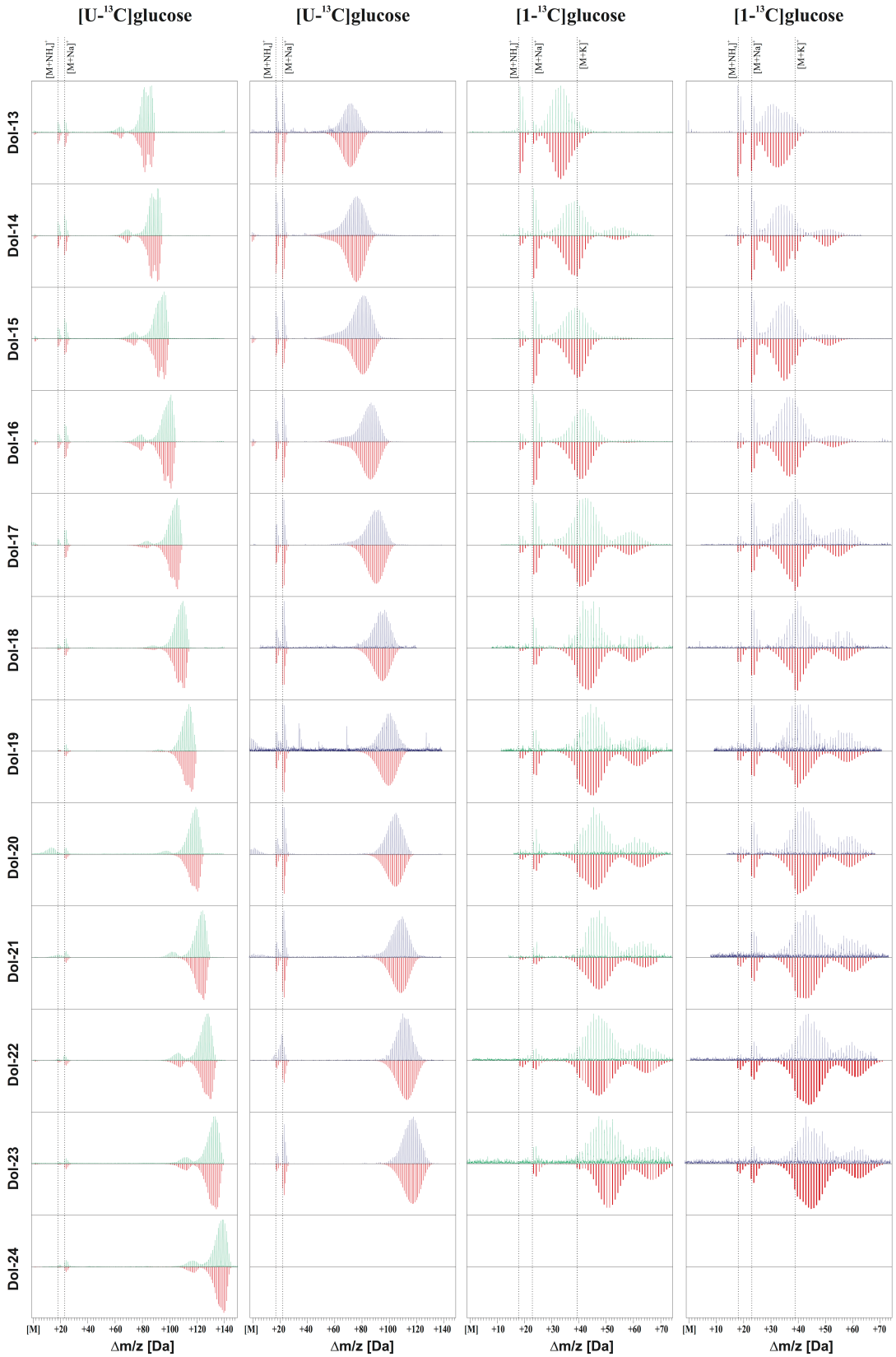
In order to evaluate the oxidative status of the hairy root treated with the oxidizing agents the level of peroxides was followed by measuring the malondialdehyde (MDA) concentration (Supplementary Fig. 2B). All of the stressors tested, *t*BHT, MV and HP, increased the MDA content (to 250%, 200% and 140% of control, respectively).

Thus, at least some of the effects of osmotic and heavy metal stresses on hairy root metabolism investigated in the main body of the present study could be mediated by ROS.



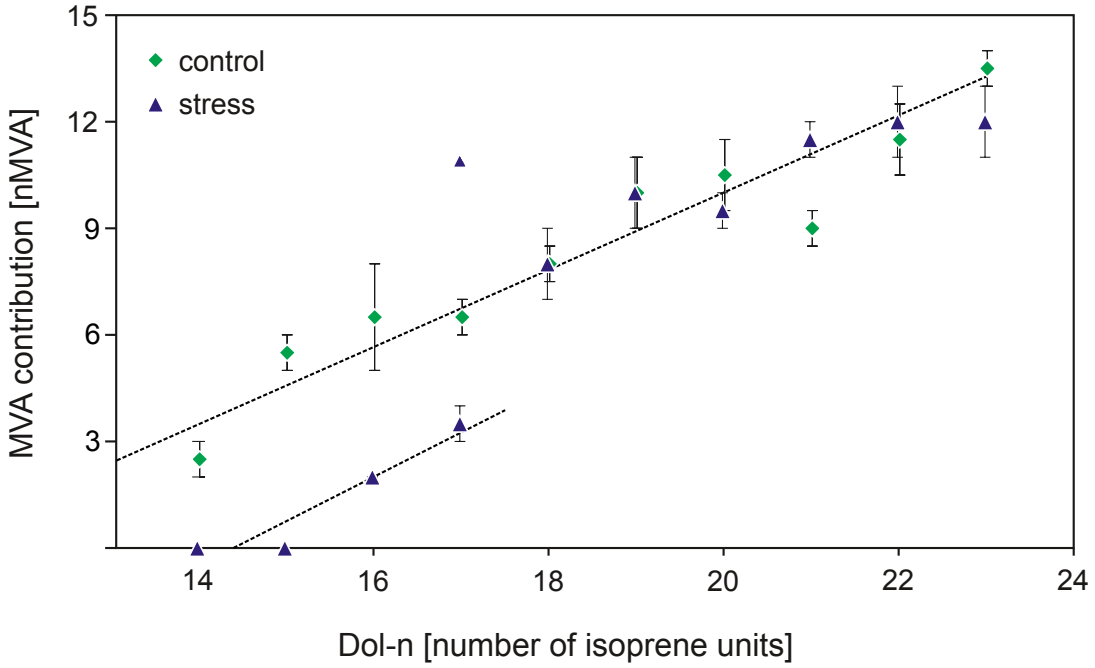
Supplementary Figure 2
Effect of oxidative stress on: (A) expression level of *CPTs* in Arabidopsis hairy roots; (B) level of MDA.

Supplementary Figure 3



Supplementary Figure 3
Experimental and theoretical spectra of [^{13}C]Dol-n after feeding [U-$^{13}C_6$]glucose (two leftmost columns) or [1-^{13}C]glucose (two rightmost columns). Theoretical spectra are shown in red, experimental in green (control) or blue (osmotic stress).
[U-$^{13}C_6$]glucose: comparison of the theoretical spectra with experimental ones allowed calculation of the effective ^{13}C abundance of 93.9 ± 1.0% under control conditions and 80.7 ± 1.3% under osmotic stress;
[1-^{13}C]glucose: comparison of the theoretical spectra with experimental ones allowed calculation of the contribution of the MEP and MVA pathways to Dol biosynthesis.
Dotted lines indicate the *m/z* of ammoniated ($[M+NH_4]^+$), sodiated ($[M+Na]^+$) and potassiated ($[M+K]^+$) ions of monoisotopic [$^{12}C_{5n}$]Dol-n.
Please note that the *m/z* axis is adjusted to the value of (M) for each respective Dol-n.

Supplementary Figure 4

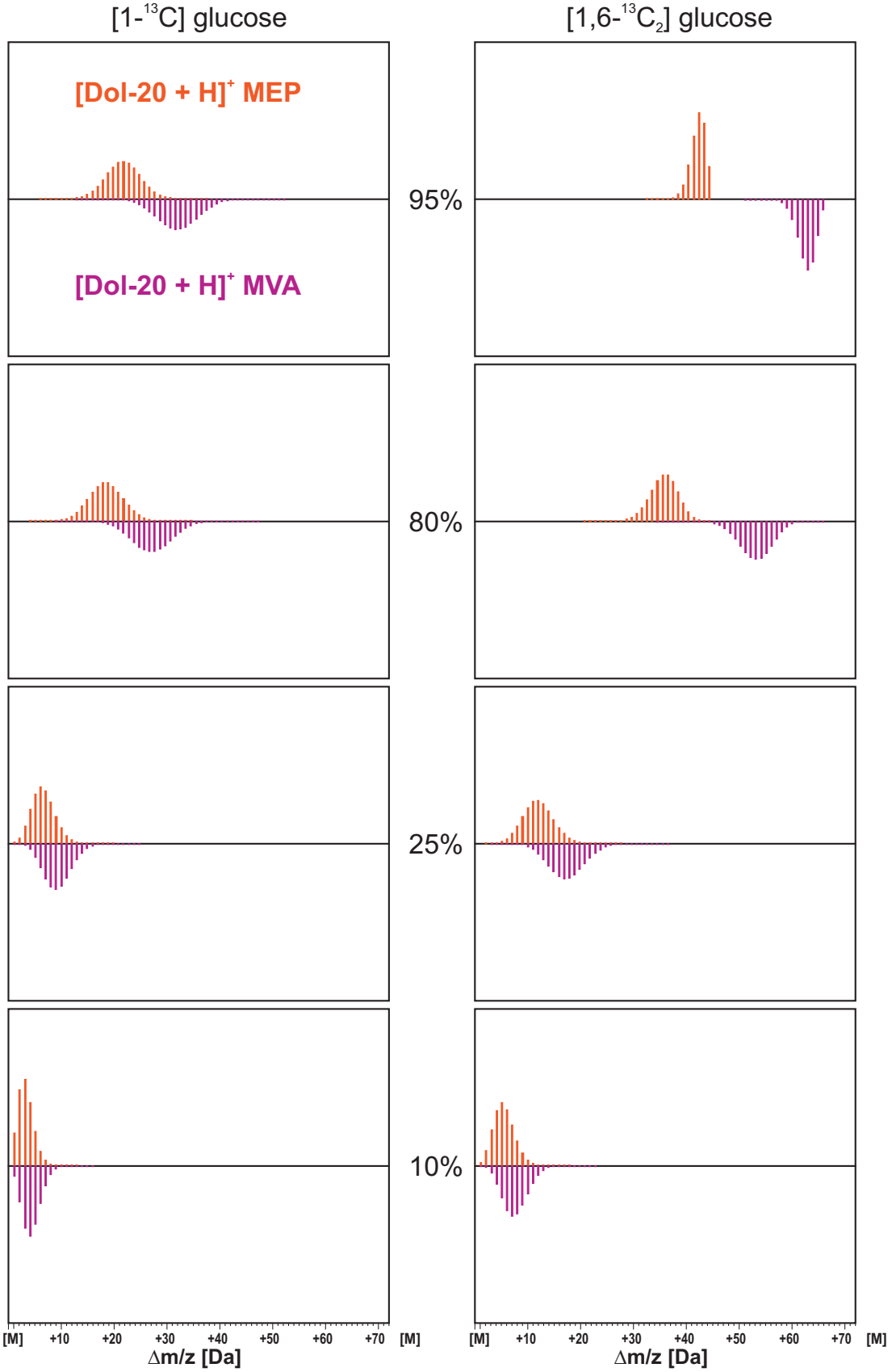


Supplementary Figure 4

Estimated number of MVA-derived isoprene units (i.u.) in various Dol-n synthesized in control (diamonds) or osmotic stress (triangles) conditions (related to Table 2).

Contribution of the MVA pathway to Dol-n biosynthesis is substantially decreased for Dol-14 -17 in the stress conditions. The metabolic origin of longer-chain Dols (Dol-18 – 21) is unaffected by sorbitol.

Supplementary Figure 5



Supplementary Figure 5

Comparison of theoretical mass spectra of Dol-20 calculated for the MEP (orange) and MVA (magenta) pathways obtained from labeling with either [1-^{13}C]glucose or [1,6-$^{13}C_2$]glucose (left and right column, respectively) of various isotopic ^{13}C abundance (95, 80, 25, 10%); shown are protonated $[M+H]^+$ ions.

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